Docket No. 56778 (70820)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT:	Y. Sasaki et al.		
U.S.S.N.:	10/019,743	GROUP:	1653
FILED:	December 28, 2001	EXAMINER:	A. U. Desai

FOR: PROCESS FOR PREPARING LH-RH DERIVATIVES

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 CFR 1.131

The undersigned declare as follows:

1. We are co-inventors of the above-identified patent application assigned to the Takeda Chemical Industries, Ltd.

2. Prior to December 18, 1998, we made and successfully isolated Purified leuprorelin or a salt thereof having a sum of all impurities of less than about 1% as disclosed and claimed in the above-identified patent application.

3. That the purified leuprorelin we made and isolated prior to December 18, 1998 also included purified compositions in which the content of 5-oxo-Pro-D-His-Trp-Ser-Tyr-D-Leu-Leu-Arg-Pro-NH-CH_2-CH_3 or a salt thereof was about 0.3% or less.

4. That the purified leuprorelin we made and isolated prior to December 18, 1998 also included purified leoprorelin compositions in which the impurities were racemic isomers of the LH-RH derivatives and/or highly polar related substances.

5. Attached as Exhibit 1 is a true and accurate copy of laboratory notebook records, as well as partial English translations of the notebook records, with dates deleted. The notebook records demonstrate that the purified leuprorelins as described in paragraphs 2, 3, and 4 above were made prior to December 18, 1998. Exhibit 1 shows, among other things, methods of characterization of the purified leuprorelin compositions, confirmation of reproducibility by scale-up, and analytical analysis for the purified leuprorelin as described in paragraphs 2, 3, and 4 above. On that Exhibit 1, the references to "S3-TAP and TAP-144" designate the particular purified leuprorelin compositions having less than 1 % impurities.

5. We hereby further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both (18 U.S.C. 1001), and that such willful false statements may jeopardize the validity of the above-identified application or any patent issued thereon.

Date: December 17, 2004 Y. Sasaki
Yasuhiro Sasaki

Date: December 17, 2004 K. Shimizu
Katsuji Shimizu

Exhibit A

メタンスルホン酸 ×1.25
発酵設後(15℃)にて HIS 抑制 部栄確認も調製

1. 300ml 4 容フルベン
2. メタンスルホン酸 178+基6 (1.25)
3. イオン水 13.4g
4. 滅菌 g
5. HBS-TAP 採取
6. 〃 保存 (2℃以下)
7. 発酵 g
(72Hr)
8. 終了
9. 発酵液を一定量ロ過年に通年仕様利用4種類
酵母 酢酸4種ml ロ過する
10. 30' 撹拌 10' 静置 アルカリ及び酸性後採取
11. アルカリ洗浄 稀釈液添加
12. pH 調整 酢酸4 89ml 添加
13. 10' 撹拌 30' 静置 水分採取
14. 水分を pH 2.4 ml 下次に全量回収 8% 食塩
10' 撹拌 30' 静置 水分を採取
15. 外に出して 遠心

1. 風袋 513.39 g
2. 活用
3.
4. 8:42 → (14.95g)
5. 12.87 g → (TAP 11.0 g 相当)
6. (8:55 ~ 9:07 (8.0)) (Temp センサ 約 8.9 内 10.3 ~ 10.5)
7. 9:07 (9.0) 水分 揮発 ゼオ 急結 ~~~ Temp
10:08' 発酵 終了 8:12:08 (10.5℃)
(木) (75.163 / 75.102) (238.2g → 7.6g) f=0.9974 → 遠心
9. 12:08 ~ 12:32 (10' ~ 5℃) (以上 不用 とする)
10. 12:32 ~ 13:02 ~ 13:12 (7.0) (5.0) (6.0) 水分 769.04 g
11. 13:16 ~ 13:20 (6.0) (6.0)
12. 5.01 → 4.49 (酢酸 8.5ml)
13. 13:25 ~ 13:35 ~ 14:05 (6.1) (5.0) (15.0) 水分 652.76 g
14. 14:13 ~ 14:23 ~ 14:53 (7.0) (3.0) (5.0)
(木) (679.28 / 178.26) f=0.9984 f=0.9958 → 遠心後
15 15:10 ~ 15:40 (25) (22)
615.16 / 613.96 f=0.9980

残存 0.00768 / 11.0
0.07%

Number of sheets & charts glued:	Number of photos glued:	Recorded by:	Date (mm/dd/yy) / /
Read and understood by:	Date (mm/dd/yy) / /	Read and understood by:	Date (mm/dd/yy) / /

○ MBS-TAP (粉) … 現場品 Lot 076 (P: 92.87%)

○ 系列 0.1%水溶液 沈降 (30.76 g)

○ 後酢酸 pH 8.21 → 4.08 (酢酸 11.5 ml)

5

残 MBS-TAP

0.0177% 0.0422 g / 11.95 = 0.35%

10 収率 4.23% 10.085 / 11.0 = 91.68%

残渣	91.68%		
凝結剤	18.72%		
1次	85.34%		
2次	87.61%	1/3	1.58% 88.73%

15

顆粒物質 (USP No)

	D-Trp	D-Ser	D-His	L-Leu
残渣	1.60	0.08	0.23	0.90
凝結剤	—	—	0.25	0.76
1次	—	—	0.26	0.92
2次	—	—	0.24	0.93 ✓
沈降	—	0.01	0.25	0.94

20

25

30

Number of sheets & charts glued:	Number of photos glued:	Recorded by:	Date (mm/dd/yy): / /
Read and understood by:	Date (mm/dd/yy) / /	Read and understood by:	Date (mm/dd/yy) / /

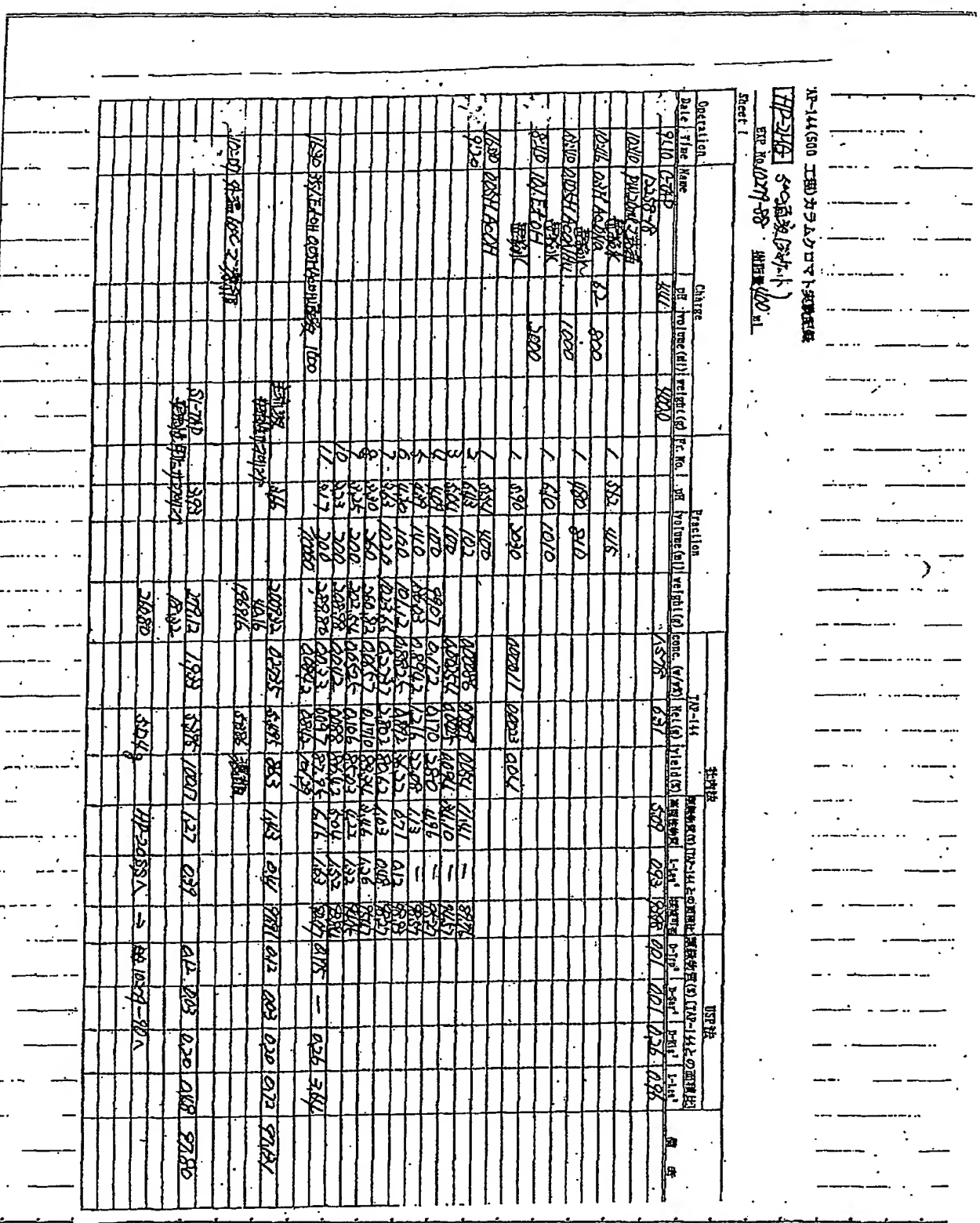
スケールUPによる再現性の確認 (1)

従来法の4工程 (HP-20 → CM-23 → HP-20 → LH-20) から 1工程削減 (HP-2HG → HP-20SS → LH-20) を目的にスケールUPにて新法を確認する。

HP-2HGによるクロマト精製 C-TAP → SI-TAP
詳細データシート参照、

結果	D-Trp体	D-Ser体	D-His体	L-Leu体	面百%	回収率
C-TAP	0.01%	0.01%	0.26%	0.96%	98.88/MAB	88.73%
SI-TAP	0.12%	0.03%	0.20%	0.68%	9888/9780	86.45%

Number of sheets & charts glued:	Number of photos glued:	Recorded by:	Date (mm/dd/yy) / /
Read and understood by:	Date (mm/dd/yy) / /	Read and understood by:	Date (mm/dd/yy) / /



Number of sheets & charts glued:	Number of photos glued:	Recorded by:	Date (mm/ dd/ yy) / /
Read and understood by:	Date (mm/ dd/ yy) / /	Read and understood by:	Date (mm/ dd/ yy) / /

HP-20SSによるSt-TAPの精製

St-TAP 10279-88

HP-20SSの通液量は 15°C 樹脂量 2容増の175ml

詳細データシート参照、

	D-Trp体	D-Ser体	D-His体	L-Leu体	面百%	回収率
結果 St-TAP	0.13	0.04	0.20	不検出	99.54/99.46	90.3

Number of sheets & charts glued:	Number of photos glued:	Recorded by:	Date (mm/ dd/ yy) / /
Read and understood by:	Date (mm/ dd/ yy) / /	Read and understood by:	Date (mm/ dd/ yy) / /

TAP-144(500 工程)カラムクロマト実験記録

HP-20SS 温度 15°C

EXP No. 10279-90 樹脂量 175 ml ※樹脂量 2割増

Sheet 1 SI-TAP lot 10279-88

Operation Date	Time	Name	Charge pH	Charge volume (ml)	Charge weight (g)	Fraction Fr. No.	Fraction pH	Fraction volume (ml)	Fraction weight (g)	社内法 TAP-144 conc. (w/w%)	社内法 TAP-144 Net (g)	社内法 TAP-144 yield (%)	社内法 類縁物質(%) [TAP-144との面積比] 高関性物質	社内法 類縁物質(%) L-Leu	社内法 類縁物質(%) 純度	USP法 類縁物質(%) [TAP-144との面積比] D-Trp	USP法 D-Ser	USP法 D-Nip	USP法 L-Leu	備考
9/15		SI-TAP	2.90		360.01															
		PW洗浄		20ml						1.883	4.90	100	1.31	0.43		0.13	0.04	0.20	0.45	
	11:30	溶離液																		
	11:30	0.1M AcONa	6.24	525ml		1	3.23	280												
	14:45	0.1M AcONH4		805		1	4.58	520												
	9:25	PW洗浄		175		1	6.04	810		2.1×10⁻⁵	1.7×10⁻⁴									
	10:45	溶離液				1	6.35	175		3.7×10⁻⁵	6.5×10⁻⁵									
	10:45	20%EtOH 溶離液		200																
	15:00					1	4.98	245		1.39×10⁻⁵	4.78×10⁻⁵									
	15:25					2	4.41	100		1.52×10⁻⁵	1.47×10⁻⁵									
	15:35					3	4.15	50												
	16:20					4	4.31	50		3.44×10⁻⁵	1.72×10⁻⁵									
	16:50					5	4.48	50		9.66×10⁻⁵	4.83×10⁻⁵	0.006								
	17:15					6	4.59	50	47.53	1.46×10⁻³	0.01	0.02								
						7	5.05	50	47.85	0.02	0.06	0.23	9.53							
						8	6.21	30	23.56	0.13	0.064	0.96	1.83							
						9	6.55	30	28.00	0.228	0.064	2.26	1.94			0.59	0.17	0.18	=	
						10	6.82	16.5	15.40	4.392	0.065	3.18	1.20			0.53	0.13	0.19	=	
	9:05	35%EtOH														0.34	0.07	×	—	
	10:05																			
	12:00				主流	1	7.83	245	382.41	1.048	3.485	94.31	0.26							
	12:55					2	8.89	120	114.56	0.824	0.085	84.21	0.37		99.04	0.14	0.03	0.19		
						3	8.59	80	73.06	0.466	0.344	91.22	0.42		99.04	0.09	0.04	0.21		
						4	8.27	25	22.17	0.485	0.107	99.41	0.44		99.13	0.10	0.06	~~0.25~~ 0.21		
						5	8.13	20	19.39	0.488	0.095	95.34	0.50		99.06	0.10	0.07	~~0.33~~ 0.25		
						6	7.14	20	17.06	0.425	0.074	95.85	2.85		99.42	0.09	0.09	0.33		
						7	6.25	21	18.82	0.262	0.049	97.86	6.47		98.97	0.12	0.39	0.08		
															99.29	0.10	0.59	0.72		
		SI-TAP主流液 35%EtOH Fr-1~Fr-4 回収率 90.23				主流液			533.94	0.815	4.354	88.86	0.47		99.53	0.13	0.04	0.20		0.5階5% 99.45
				収 4.429 / 4.429		SI-TAP			30.01	14.145	4.24	86.63	0.46		99.54	0.13	0.04	0.20		99.46
						オフ溶離液			23.58		3.90									
												F5279-96A	HC20							

Number of sheets & charts glued:	Number of photos glued:	Recorded by:	Date (mm/dd/yy) / /
Read and understood by:	Date (mm/dd/yy) / /	Read and understood by:	Date (mm/dd/yy) / /

LH-20 による精製

S2-TAP　10209-90

通液条件は現行法

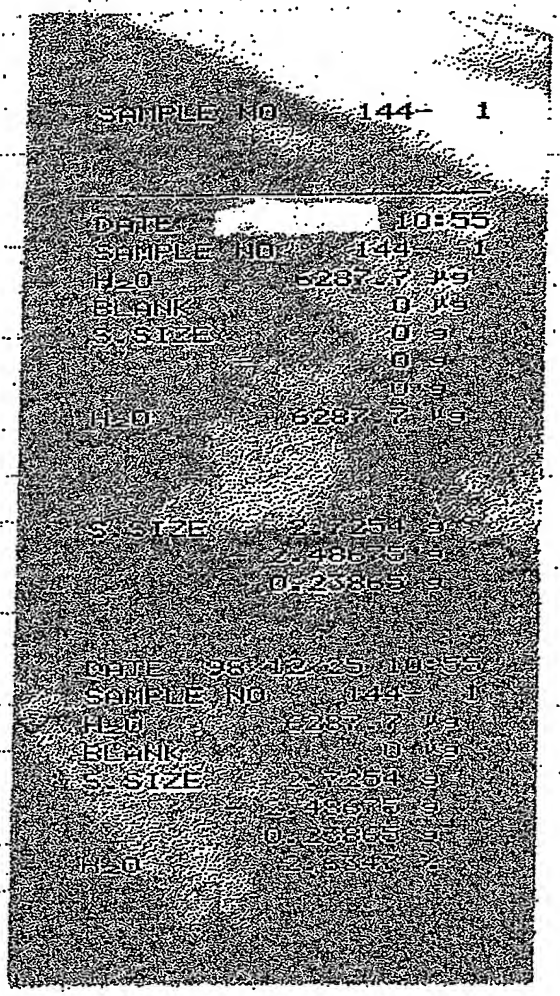
詳細データシート参照、

結果

	D-Trp体	D-Ser体	D-His体	L-Leu体	面百%	回収率
S3-TAP	0.13	0.04	0.20	N.D	99.54/99.46	90.23
S4-TAP	0.13	0.04	0.20	N.D	99.54/99.42	97.21
製品	0.14	0.05	0.19	N.D	99.52/99.45	

水分測定

<SAMPLE NO.> 01-65
LOT NO. 000
FACTOR
BUR1 2.041 MG/ML
SIZE
B.G. 0.0 MICR 0
WT1 15.92153 G
WT2 15.63017 G
RESULT 3.432 %
BUR1 3.520 ML



TAP-144(500 工程)カラムクロマト実験記録

LH-20 室温

EXP No. 1027996　樹脂量 145 ml

Sheet 1

Operation Date	Operation Time	Name	Charge pH	Charge volume(ml)	Charge weight(g)	Fraction Fr. No.	Fraction pH	Fraction volume(ml)	Fraction weight(g)	社内法 TAP-144 conc. (w/w%)	社内法 TAP-144 Nel(g)	社内法 TAP-144 yield(%)	社内法 類縁物質(%)[TAP-144との面積比] 高極性物質	社内法 類縁物質(%)[TAP-144との面積比] L-Leu	社内法 類縁物質(%)[TAP-144との面積比] 検出可否	USP法 類縁物質(%)[TAP-144との面積比] D-Trp	USP法 類縁物質(%)[TAP-144との面積比] D-Ser	USP法 類縁物質(%)[TAP-144との面積比] D-His	USP法 類縁物質(%)[TAP-144との面積比] L-Leu	備考
	10:20	S2TAP	2.70		27.42					14.44	3.96		0.45			0.13	0.04	0.19	—	
		溶解				1	6.21	25		0.0018	0.00041	0.01	6.07	4.827						
	12:00	0.075N AcOH	3.56	435																
	14:00																			
						1	5.85	30	29.86	0.000051	2.5-05	0.011	140.91							
						2	5.75	15	13.91	0.000057	9.4-07	0.011	754.11							
						3	—	5.5	5.08	0.000041	2.3-06	0.011	208.80							
9/20						4	5.65	10	9.52	0.000058	6.5-06	0.011	38.70							
	10:05					5	5.63	10	9.45	—	—	0.011	—							
	10:45					6	5.30	10	9.36	—	—	0.011	—							
	11:25					7	6.50	11	10.44	2.460	0.257	2.49	0.53			0.03	0.03	0.06	—	
						8	7.21	60	58.93	5.310	3.131	85.64	0.32	0.327		0.13	0.05	0.21	—	
						9	6.90	25	23.91	1.670	0.400	95.64	0.58			0.20	0.03	0.25	—	
						10	6.70	15	14.09	0.771	0.112	98.41	0.24			0.20	0.03	0.23	—	USP面積%
		S2TAP (1) Fr-8,9 Y=87.15% ※98.58%					7.24		80.09	4.17	3.277	83.27	0.46		98.54	0.14	0.04	0.21	—	99.39%
		S3 (2) Fr-7,8,9,10 Y=98.05% ※101.08					7.13		101.76	3.573	3.636	91.50	0.60		98.41	0.13	0.04	0.20	—	98.38%
				脱塩液 0.079g(Na)		…	ロス有り				(3.350)	(97.21)								
		S2TAP 濃縮液					6.49		33.0	10.18	0.360	84.84	0.48		98.53	0.13	0.04	0.20		99.42
	10:00 ~ 17:00	濃縮乾燥		99.63 − 66.63																
				33.00 − 0.459 残			1/5 JP系		343											
							USP系		264											
				32.55g																

Number of sheets & charts glued:	Number of photos glued:	Recorded by:	Date (mm/ dd/ yy) / /
Read and understood by:	Date (mm/ dd/ yy) / /	Read and understood by:	Date (mm/ dd/ yy) / /

Partial English Translation of Laboratory Notebook Records

Page 18 (12258-18)

Methane sulfonic acid x 1.25
Confirmation of inhibition of His effect and preparation ~~at reaction temp. 15°C~~

1. 300 ml 4-neck flask	1. tare 513.39 g
2. methane sulfonic acid 17.8+ 32.3 (1.25)	2. including washing
3. phenol 13.4 g	3.
4. cooling S	4. 8:42
5. NBS-TAP weighing	5. 12.87 g (corr . to TAP 11.0 g)
6. ditto charge (8°C or lower)	6. 8:55 (7.0) - 9:07 (8.0)
7. reaction S (3 Hr)	7. 9:07 (9.0)
8. finished	8. 12:08
.....	.....
15. concentrated at 25°C of outer temp.	15. 15:10 (25)-15:40 (25)
	615.16
	613.96 f=0.9980
	aqueous layer loss
	0.0076g/H_2O
	0.07%

・MBS・TAP (dry) plant product Lot076 (P: 92.87%)
・potassium carbonate 0.25 min. additional dissolution (30.76 g)
・buffer pH 8.21 → 4.08 (acetic acid 11.5 ml)

remainder MBS・TAP
0.0177% 0.0422 g/11.95 = 0.35%

reaction rate 4.23% 10.085/11.0 = 91.68%

reaction mixture	91.68%		
before concentration	78.72%		
after concentration	85.34%		
after overnight	87.61%	/.58%	88.73%

related substances (USP method)

	D-Trp	D-Ser	D-His	L-Leu
reaction mixture	1.60	0.08	0.23	0.90
before conc.	-	-	0.25	0.76
after conc.	-	-	0.26	0.92
after overnight	-	-	0.24	0.93
refrigeration		0.01	0.25	0.94

Confirmation of reproducibility by scale-up (1)

For the purpose of reducing one column (HP-2MG→HP-20SS→LH-20) from the previous 4 columns (HP-20→ CH-23→HP-20→LH-20), the new process is confirmed by scale-up.

chromatographic purification by HP-2MG C-TAP→S1-TAP
cf. detailed data sheet

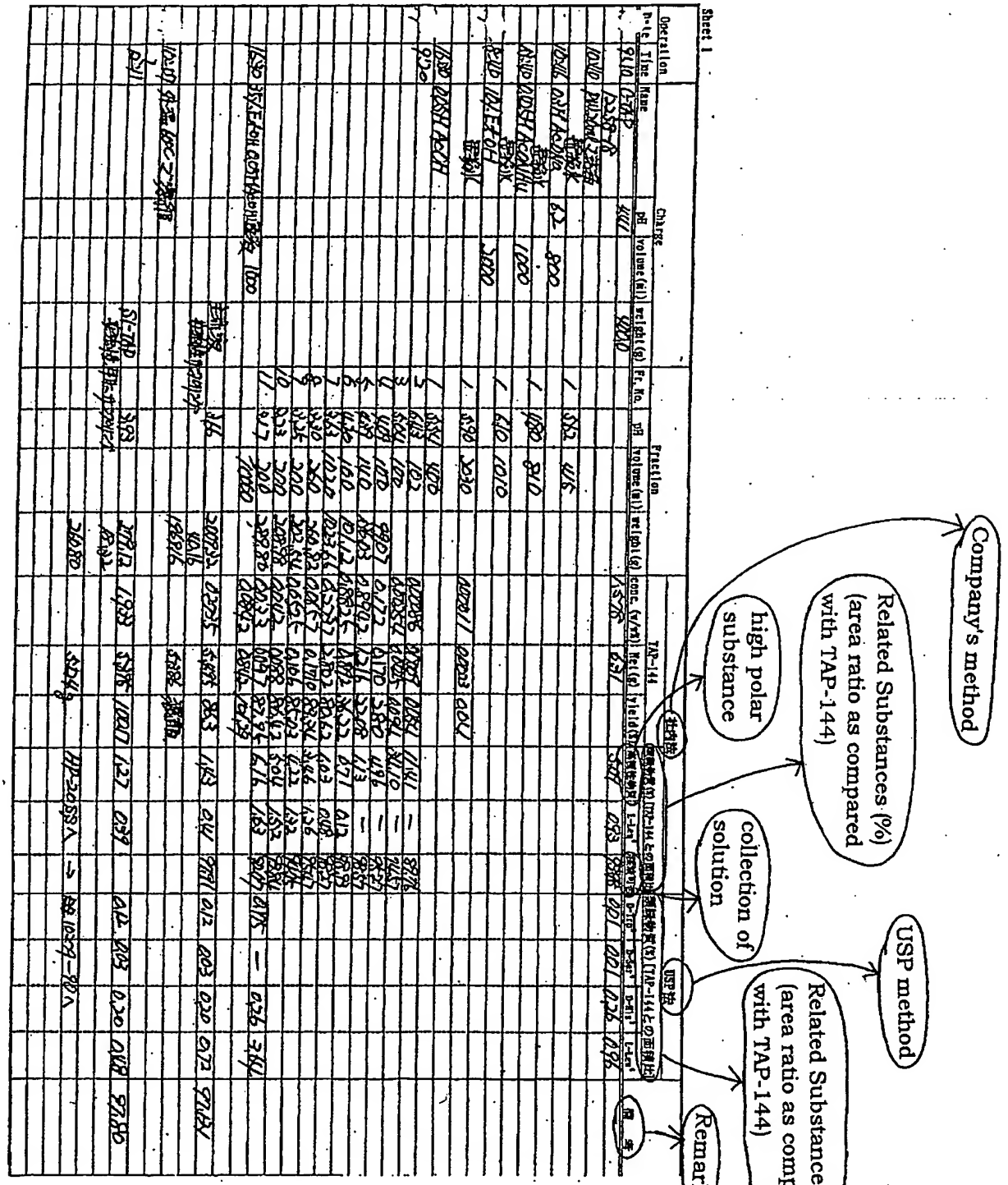
Results

	D-Trp^{3} form	D-Ser^{4} form	D-His^{2} form	L-Leu^{6} form
C-TAP	0.01%	0.01%	0.26%	0.96%
S1-TAP	0.12%	0.03%	0.20%	0.48%

	area %	recovery
C-TAP	93.98/77.43	88.73%
S1-TAP	98.08/97.80	86.45%

Takeda Chemical Industries, Ltd.

AP-144 (500 Step) Column Chromatography Experimental Record
HP-2MG 5° C passage (jacket)
EXP No. 10279-88 resin amount 400 ml

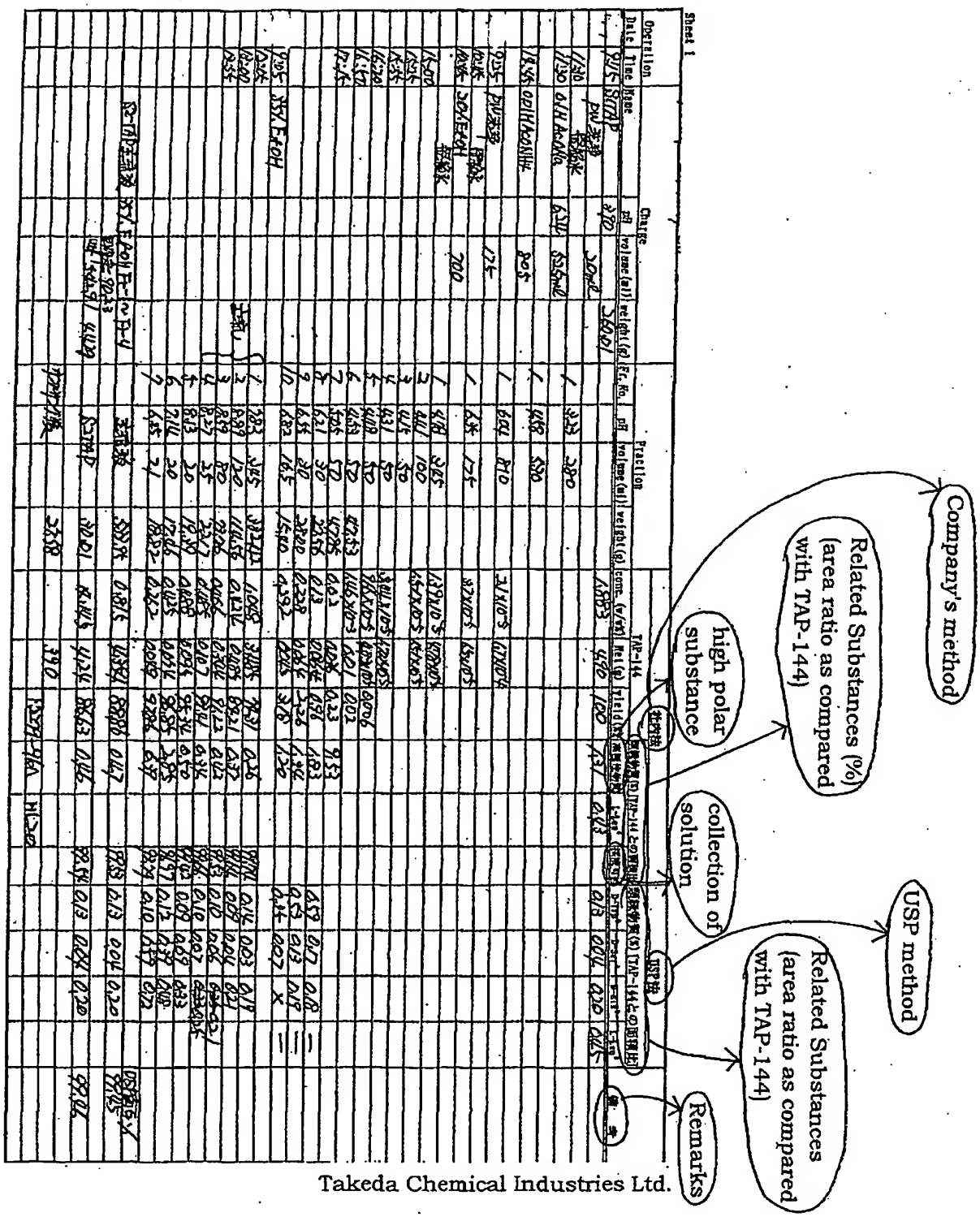


Purification of S1-TAP by HP-20SS
S1-TAP 10279-88
HP-20SS passage temperature 15°C, resin amount 20% up 175 ml
cf. detailed data sheet

Results

	D-Trp[3] form	D-Ser[4] form	D-His[2] form	L-Leu form
S2-TAP	0.13	0.04	0.20	not detected
	area %	recovery		
S2-TAP	99.54/99.45	90.23		

AP-144 (500 Step) Column Chromatography Experimental Record
HP-20SS Temp. 15°C
EXP No. 10279-90 resin amount 175 ml *resin amount 20% up
S1-TAP Lot 10279-88



Purification by LH-20

S2-TAP 10279-90

Passage conditions is the current method.

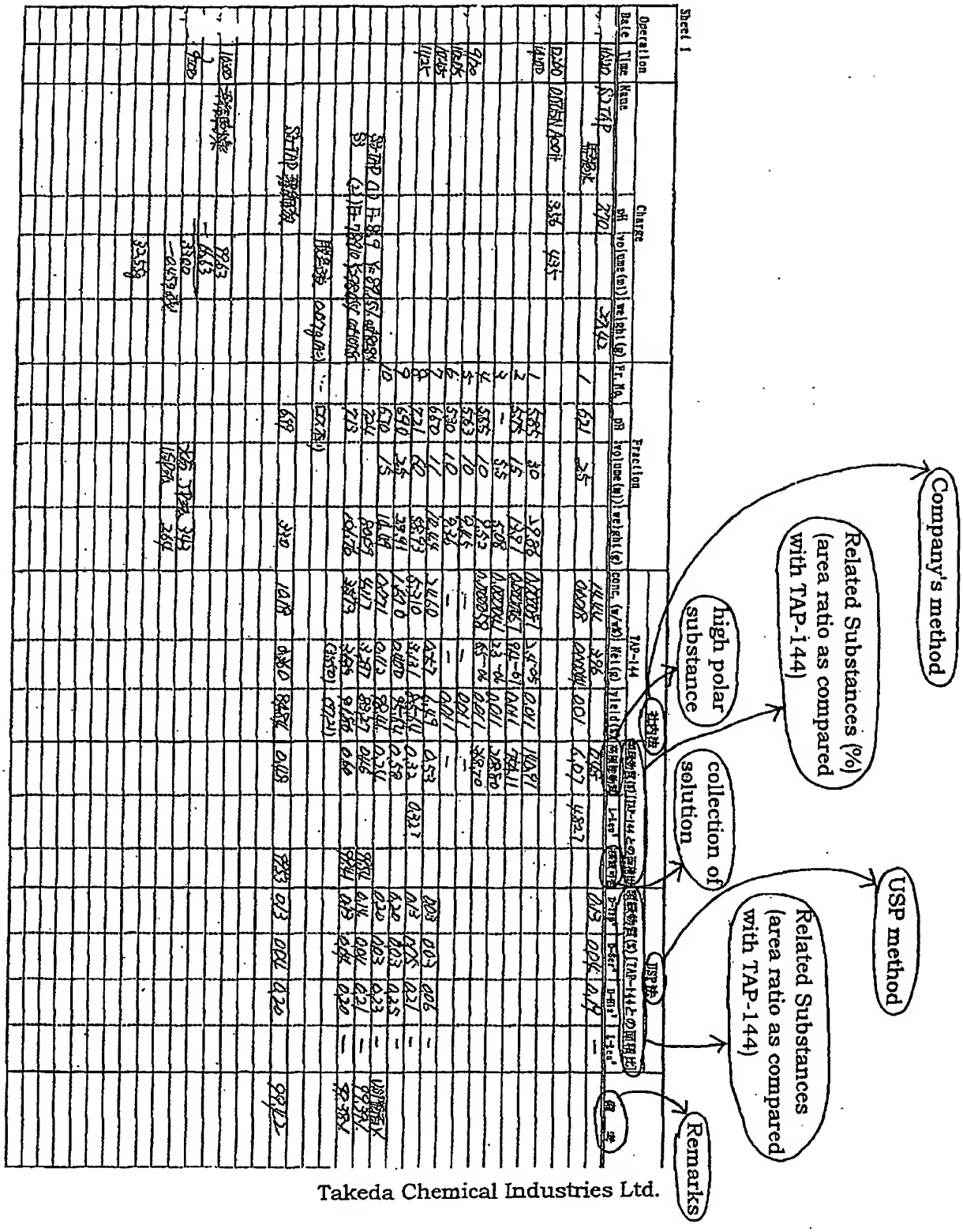
cf. detailed data sheet

Results

	D-Trp^{3} form	D-Ser^{4} form	D-His form	L-Leu^{6} form
S3-TAP	0.13	0.04	0.20	N·D
S4-TAP	0.13	0.04	0.20	N·D
product	0.14	0.05	0.19	N·D
	area %	recovery		
S3-TAP	99.54/99.46	90.23		
S4-TAP	99.54/99.42	97.21		
product	99.52/99.45			

Moisture Measurement

AP-144 (500 Step) Column Chromatography Experimental Record
LH-20 room temp.
EXP No. 10279-96 resin amount 145 ml



Summary of Records

Re: Details of obtaining a solution containing TAP-144 whose impurities content is 1% or lower and D-His^2 form content is 0.3% or lower

D-His^2 form content has been already reduced to 0.3% or lower by studying de-MBS reaction conditions before purification (C-TAP), and then, impurities in TAP-144 is reduced to 1.0% or lower by chromatographic purification.

Date	Steps	Experimental method	Results	Notebook No.
	MBSTAP ↓ C-TAB (corr. to Example 1)	Purpose: To confirm effect on control of D-His^2 form. Experimental method Amount of MESE charge: 1.25-fold Reaction time: 3 hrs Reaction temp.: 11°C	USP method area % D-His^2 form: 0.25%	12258-18 to 19
	C-TAP ↓ S1-TAP (corr. to Example 2)	Purpose: To purify C-TAP by HP2MG. Experimental method Passage temp.: 5°C (jacket) Resin amount: 400 ml C-TAP amount: 6.31 g Solvent: 0.05M AcOH	USP method area % S1-TAP D-His^2 form: 0.26%→ 0.20% TAP-144: 97.80%	10279-88 to 89
	S1-TAP ↓ S3-TAP (corr. to Example 3)	Purpose: To purify S1-TAP by HP-20SS Experimental method Passage temp.: 15°C (jacket) Resin amount: 175 ml C-TAP amount: 4.90g Solvent: 20% EtOH→ 35% EtOH	USP method area % S3-TAP D-His^2 form: 0.20%→ 0.20% TAP-144: 97.80%→ 99.46%	10279-90 to 91
	S3-TAP ↓ S4-TAP (corr. to Example 4)	Purpose: To purify S3-TAP by LH-20 Experimental method (current method) Passage temp.: room temp. Resin amount: 145 ml C-TAP amount: 3.96g Solvent: 0.005N AcOH	USP method area % S4-TAP D-His^2 form: 0.20%→ 0.20% TAP-144: 99.46%→ 99.42%	10279-96 to 97

Abbreviations in Notebook	Examples in the Present Application
MBSTAP	5-oxo-L-propyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-D-leucyl-L-leucyl-Nw-p-methoxybenzenesulfonyl-L-arginyl-N-ethyl-L-prolinamide
C-TAP	Aqueous solution (1) of Leuprolide
S1-TAP	Aqueous solution (2) of Leuprolide
S3-TAP	Aqueous solution (3) of Leuprolide
S4-TAP	Concentrated fractions containing leuprolide eluted with a 0.005 M aqueous solution of acetic acid